2′-Modified Nucleosides for Site-Specific Labeling of Oligonucleotides

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We report the synthesis of 2′-modified nucleosides designed specifically for incorporating labels into oligonucleotides. Conversion of these nucleosides to phosphoramidite and solid support-bound derivatives proceeds in good yield. Large-scale synthesis of 11-mer oligonucleotides possessing the 2′-modified nucleosides is achieved using these derivatives. Thermal denaturation studies indicate that the presence of 2′-modified nucleosides in 11-mer duplexes has minimal destabilizing effects on the duplex structure when the nucleosides are placed at the duplex termini. The powerful combination of phosphoramidite and support-bound derivatives of 2′-modified nucleosides affords the large-scale preparation of an entirely new class of oligonucleotides. The ability to synthesize oligonucleotides containing label attachment sites at 3′, intervening, and 5′ locations of a duplex is a significant advance in the development of oligonucleotide conjugates.

INTRODUCTION

Substituents at the 2′-ribose position of nucleosides are important structural and mechanistic probes of nuclease resistance and ribozyme catalysis (*1*). These nucleosides are used in the development of antisense therapeutics (*1b*) and the rapid screening of oligonucleotide sequences displaying high affinity toward protein targets (*2*). Nucleosides containing a primary amine at the 2′ position facilitate the incorporation of several reporter molecules or labels into oligonucleotides (*3*). This is accomplished by (a) introducing the amine-containing nucleoside into an oligonucleotide using standard automated DNA synthesis, (b) purifying the resulting oligonucleotide, (c) reacting the reporter group with the amine-containing oligonucleotide, and (d) isolating the conjugate. Labels such as fluorescent dyes (*3a,c*), aromatic and aliphatic isocyanates (*3b,d*), and transition metal complexes (*3e*) have been successfully incorporated into oligonucleotides using this method.

Our objective is to develop new methods of incorporating labels, namely transition metal complexes, into DNA site-specifically (*4*). To this end we have designed nucleosides containing bidentate amine groups at the 2′-ribose position to which transition metal complexes are chelated (Figure 1). This site on the ribose ring is selected so that both solid support-bound and phosphoramidite forms of the nucleosides can be prepared. The solid support-bound nucleoside is used as the starting material in oligonucleotide synthesis, whereas the phosphoramidite monomer can be introduced at any later position in the oligonucleotide sequence. As a result, labels can be incorporated at the 3′, intervening, or 5′ locations of an oligonucleotide. We report the synthesis of 2′-modified nucleosides as both solid support-bound and phosphoramidite derivatives and their incorporation into oligonucleotides via solid-phase methods. Thermal denaturation studies of the resulting oligonucleotides indicate that the presence of the 2′-modified nucleosides has only a slight destabilizing effect on the duplex structure.

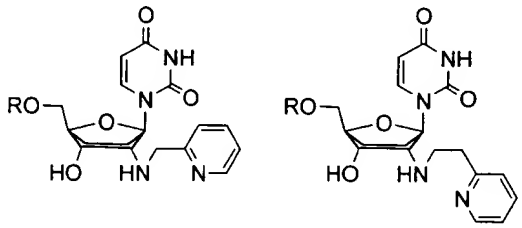

Figure 1. Structure of nucleosides with bidentate ligands at the 2′ ribose position (R = 4,4′-dimethoxytrityl).

EXPERIMENTAL PROCEDURES

General. ^1H and ^{31}P spectra were acquired on either Varian 300 or 500 spectrometers. Chemical shifts are reported in parts per million and referenced to the proton chemical shifts of deuterated solvent, trimethylsilane, or 85% H_3PO_4 in H_2O. Reagents and starting materials were used as received from Aldrich. Flash chromatography was performed on EM Science/Merck silica gel 60 (230–400 mesh). Thin-layer chromatography (TLC) was performed on 0.25 mm Merck precoated silica plates (60 F_{254}). Enzymes were purchased from Pharmacia. Mass spectrometry was performed at the Caltech Peptide/Protein Microanalytical Laboratory.

5′-*O*-(4,4′-Dimethoxytrityl)-2,2′-*O*-anhydro-1-(β-D-arabinofuranosyl)uracil, 1. This compound was prepared from the reaction of 2,2′-*O*-anhydro-1-(β-D-arabinofuranosyl)uracil and 4,4′-dimethoxytrityl chloride as previously described (*5*).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*N*,3′-*O*-(2-oxooxazolidinyl)-2′-aminomethylpyridyl-2′-deoxyuridine, 2a. To a solution of **1** (2.2 g, 4.2 mmol) in pyridine (40 mL) was added 1,1′-carbonyldiimidazole (1.01 g, 6.2 mmol). After 30 h of stirring at ambient temperature, the solvent was removed and the residue was resuspended in dichloromethane (40 mL); DIEA (1.1 mL, 6.3 mmol) and AMPy (649 μL, 6.3 mmol) were delivered to the solution. After 60 h of stirring at ambient temperature, the reaction was quenched with 5% citric acid, extracted with fresh dichloromethane, dried over Na_2SO_4, and concentrated

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to an oil. The residue was dissolved in tetrahydrofuran (25 mL), DBU (628 μL, 4.2 mmol) was added to the flask, and the solution was refluxed for 46 h. The solvent was removed, and the residue was purified on silica (using 22% EtOAc in dichloromethane containing 1% TEA and 3% methanol) to afford **2a** in 20% yield (548 mg, 828 μmol). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.48–3.58 (m, 2H), 3.78 (s, 6H), 4.35–4.40 (m, 1H), 4.54 (d, 1H), 4.75 (dd, 2H), 5.14–5.17 (m, 1H), 5.41 (d, 1H), 6.06 (d, 1H), 6.82 (d, 4H), 7.21–7.30 (m, 9H), 7.33–7.37 (m, 2H), 7.53 (d, 1H), 7.69 (t, 1H), 8.51 (d, 1H), 8.84 (s, 1H). ESI-MS mass calcd for $C_{37}H_{35}N_4O_8$ $(M + H)^+$: 663.24. Found: 663.2.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*N*,3′-*O*-(2-oxooxazolidinyl)-2′-aminoethylpyridyl-2′-deoxyuridine, 2b. To a solution of **1** (1.0 g, 1.9 mmol) in pyridine (30 mL) was added 1,1′-carbonyldiimidazole (0.460 g, 2.8 mmol). After 30 h of stirring at ambient temperature, the solvent was removed by rotary evaporation and the residue was resuspended in dichloromethane (30 mL); DIEA (500 μL, 2.85 mmol) and AEPy (340 μL, 2.85 mmol) were delivered to the solution. After 60 h of stirring at ambient temperature, the reaction was worked up as described for **2a**. The residue was dissolved in THF (11 mL), DBU (284 μL, 1.9 mmol) was added to the flask, and the solution was refluxed for 46 h. The solvent was removed by rotary evaporation, and the residue was purified on silica (eluting with 5–15% methanol in EtOAc) to afford **2b** in 10% yield (127 mg, 188 μmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.07–3.39 (m, 2H), 3.43–3.60 (m, 2H), 3.74–3.77 (m, 6H), 3.93–4.04 (m, 1H), 4.26–4.31 (m, 1H), 4.64 (dd, 2H), 5.09–5.14 (m, 1H), 5.35 (d, 1H), 6.09 (d, 1H), 6.84 (d, 4H), 7.15–7.39 (m, 11H), 7.59–7.69 (m, 2H), 8.57 (d, 1H). ESI-MS mass calcd for $C_{38}H_{37}N_4O_8$ $(M + H)^+$: 677.25. Found: 677.2.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-aminomethylpyridyl-2′-deoxyuridine, 3a. Compound **2a** (242 mg, 0.36 mmol) was suspended in dioxane (6 mL), 4 M NaOH (4.7 mL), and methanol (4.7 mL), and the mixture was stirred at 50 °C for 36 h. The solvents were removed by rotary evaporation, and the residue was dissolved in dichloromethane (55 mL). The solution was extracted with brine, dried over sodium sulfate, and evaporated to dryness to give **3a** in 92% yield (216 mg, 0.34 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.3 (s(br), 4H), 3.73–3.78 (m, 6H), 3.94–4.02 (m, 1H), 4.1 (d, 1H), 4.2 (s, 1H), 5.3 (d, 1H), 6.1 (d, 1H), 6.7 (dd, 4H), 7.1–7.3 (m, 11H), 7.5–7.6 (m, 2H), 8.4 (s, 1H). ESI-MS mass calcd for $C_{36}H_{37}N_4O_7$ $(M + H)^+$: 637.26. Found: 637.2.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-aminomethylpyridyl-2′-deoxyuridine, 3b. Compound **2b** (1.7 g, 2.51 mmol) was suspended in dioxane (43 mL), 4 M NaOH (26 mL), and methanol (26 mL), and the mixture was stirred at room temperature for 20 h. The reaction was heated at 60 °C for an additional 2 h, after which the solvents were removed by rotary evaporation. The residue dissolved in dichloromethane, extracted with brine, dried over sodium sulfate, and evaporated to dryness. The material was purified on silica (eluting with 23% ethyl acetate in dichloromethane containing 1% each of methanol and triethylamine) to give **3b** in 88% yield (1.44 g, 2.21 mmol). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.03 (t, 2H), 3.12–3.25 (m, 2H), 3.43–3.53 (m, 3H), 3.80 (s, 6H), 4.29 (s, 1H), 4.49 (d, 1H), 5.37 (d, 1H), 5.94 (d, 1H), 6.14 (s(br), 1H), 6.85 (d, 4H), 7.18–7.33 (m, 10H), 7.40 (d, 1H), 7.67 (t, 1H), 7.74 (d, 1H), 8.49 (d, 1H). ESI-MS mass calcd for $C_{37}H_{39}N_4O_7$ $(M + H)^+$: 651.27. Found: 651.2.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-trifluoroacetamido-2′-deoxyuridine, 4. This compound was prepared either from reaction of 2′-amino-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine and ethyl trifluoroacetate (*5*) or from reaction *N*^{2}-trifluoroacetyl-2′-amino-2′-deoxyuridine and 4,4′-dimethoxytrityl chloride as previously described (*3a*).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-aminom thylpyridyl −2′-deoxyuridine-3′-*O*-succinate, 5. Compound **3a** (100 mg, 0.157 mmol), DMAP (9.4 mg, 0.5 equiv), and succinic anhydride (17.3 mg, 1.1 equiv) were suspended in dry pyridine (1 mL) and allowed to stir under argon for 2 h, at which time an additional 0.2 equiv of succinic anhydride was added. The reaction proceeded overnight at room temperature, after which the solvent was removed. The residue was dissolved in dichloromethane (20 mL) and stirred with an equal volume of 5% $NaHCO_3$ solution for 2 h. The mixture was partitioned, and the organic phase was extracted with brine. Each phase was back-extracted once. The combined organic phases were washed with cold 5% citric acid and concentrated to a small volume (3–5 mL). The sample was precipitated in stirring hexanes, and the white solid **5** was collected by filtration in 60% yield (69 mg, 0.094 mmol). ^{13}C NMR (75 MHz, CD_3CN) δ 178.82, 174.10, 165.67, 160.11, 158.27, 156.03, 146.0, 145.95, 136.96, 136.76, 136.6, 133.06, 131.32, 129.47, 128.77, 127.74, 122.9, 114.45, 114.0, 102.0, 88.12, 85.33, 73.0, 72.88, 65.04, 56.03, 30.98. ESI-MS mass calcd for $C_{40}H_{39}N_4O_{10}$ $(M - H)^-$: 735.27. Found: 735.2.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-trifluoroacetamido-2′-deoxyuridine-3′-*O*-succinate, 6. Compound **4** (100 mg, 0.156 mmol), DMAP (9.4 mg, 0.5 equiv), and succinic anhydride (17.2 mg, 1.1 equiv) were suspended in dry pyridine (2 mL) and stirred under argon for 2 h. An additional 0.2–0.5 equiv of succinic anhydride was added, and the reaction proceeded at room-temperature overnight. The reaction was worked according to the procedure given for **5**. A white powder **7** was isolated in 74% yield (85 mg, 0.115 mmol). ^{13}C NMR (75 MHz, CD_3CN) δ 175.04, 172.80, 163.85, 160.13, 140.71, 131.42, 130.35, 129.32, 128.96, 128.43, 114.60, 114.23, 103.97, 86.30, 84.29, 74.18, 64.77, 56.42, 55.77, 30.49. ESI-MS mass calcd for $C_{36}H_{33}N_3O_{11}$ $(M - H)^-$: 740.21. Found: 740.2.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-aminomethylpyridyl-2′-deoxyuridine-3′-*O*-succinated Support, 7. Compound **7** was prepared by suspending the solid support (controlled pore glass derivatized with long-chain alkylamine, 500 Å pore size, 350 mg) in dry dichloromethane (5 mL) and adding TEA (250 μL) and **5** (180 mg, 245 μmol); HOBT (33 mg, 245 μmol) and BOP (119 mg, 270 μmol) were added to the suspension. The mixture was agitated for 16 h at room temperature, filtered, and washed with dichloromethane (2 × 10 mL). The solid was transferred to a separate flask and suspended in pyridine (7.5 mL). Acetic anhydride (1–2 mL) and *N*-methylimidazole (100 μL) were added to the flask, and the mixture was agitated overnight. The solid was filtered and washed with pyridine (3 × 10 mL), methanol (3 × 10 mL), dichloromethane (3 × 10 mL), and diethyl ether (3 × 10 mL). The white solid **7** was dried in vacuo. The nucleoside loading was determined spectrophotometrically to be 60 μmol/g (*6*).

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-trifluoroacetamido-2′-deoxyuridine-3′-*O*-succinated Support, 8. Compound **8** was prepared using the same method described for **7**. The nucleoside loading for **8** was 52 μmol/g.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-aminomethylpyridyl-2′-deoxyuridine-3′-*O*-(2-cyanoethyl *N*,*N*-Diisopropylaminophosphoramidite), 9. While under argon **3a** (200 mg, 0.314 mmol) was dissolved in dry dichloromethane (2 mL) containing DIEA (220 μL, 4 equiv). The reaction vessel was degassed several times prior to

the addition of 2-chlorocyanoethyl *N,N*-diisopropylaminophosphoramidite (105 μL, 1.5 equiv) dropwise over 5 min. A positive ninhydrin test indicated formation of the desired product. After 50 min the reaction was diluted with 200 μL of methanol and evaporated to dryness. The residue was applied to silica (eluting with 0–80% dichloromethane in hexane containing 1% TEA) to give an off-white powder **9** in 57% yield (151 mg, 0.180 mmol). ESI-MS mass calcd for $C_{45}H_{52}N_6O_8P$ $(M - H)^-$: 835.37. Found: 835.4.

5′-*O*-(4,4′-Dimethoxytrityl)-N^2-trifluoroacetamido-2′-deoxyuridine-3′-*O*-(2-cyanoethyl *N,N*-diisopropylaminophosphoramidite), 10. While under argon **4** (100 mg, 0.163 mmol) was dissolved in dry dichloromethane (2 mL) containing DIEA (110 μL, 4 equiv). The reaction vessel was degassed several times prior to the addition of 2-chlorocyanoethyl *N,N*-diisopropylaminophosphoramidite (53 μL, 1.5 equiv) dropwise over 5 min. A positive ninhydrin test indicated formation of the desired product. After 90 min the reaction was diluted with ethyl acetate which had been previously washed with cold 10% sodium carbonate. The organic layer was extracted twice with cold 10% sodium carbonate and once with brine. The organic fraction was dried over sodium sulfate and evaporated to an oil. The residue was purified on silica (eluting with 20% hexane in dichloromethane containing 2% methanol and 1% TEA) to yield an off-white powder **10** in 77% yield (105 mg, 0.125 mmol). ^{1}H NMR (CD_3CN, 300 MHz) δ 1.26 (d, 12H), 3.4 (m, 2H), 3.5 (m, 2H), 3.6–3.8 (m, 3H), 3.79 (s, 6H), 4.1 (m, 2H), 4.3 (m, 1H), 4.4 (m, 1H), 4.5 (m, 1H), 4.7 (m, 1H), 4.8 (m, 1H), 5.4 (d, 1H), 5.8 (s, 1H), 6.1 (d, 1H), 6.9 (m, 4H), 7.3 (m, 9H), 7.5 (m, 2H), 7.6 (t, 1H), 7.9 (s, 1H). ^{31}P NMR (CD_3CN, 300 MHz) δ 153.13 and 154.06 ppm observed for the diastereomers. ESI-MS mass calcd for $C_{41}H_{46}F_3N_5O_9P$ $(M - H)^-$: 840.31. Found: 840.2.

Oligonucleotide Synthesis. Protected deoxyribonucleoside phosphoramidites and other reagents required for solid-phase DNA synthesis were purchased from Applied Biosystems, Incorporated (ABI). All oligonucleotides were synthesized on a 1.0 μmole scale. Solid supports (**7**, **8**) were packed in column assemblies purchased from ABI and contained approximately 23 mg of derivatized resin, depending on the nucleoside loading. The initial coupling steps in each synthesis were increased from 30 s to 2 min. The concentration of phosphoramidites (**9**, **10**) typically ranged from 0.1 to 0.18 M in dry acetonitrile. The coupling time for **9** and **10** was 15 min.

Oligonucleotides **11**, **13**, and **14** were synthesized with the terminal dimethoxytrityl (DMT) group retained. Oligonucleotide **12** was prepared with the 5′-DMT group removed prior to cleavage and deprotection. Oligonucleotides **11**–**13** were cleaved from the solid support with concentrated ammonia during the automated synthesis routine and deprotected for either 16 h at 55 °C or 4 h at 65 °C. Oligonucleotide **14** was cleaved manually in 5 mL of concentrated ammonia for 16 h at 55 °C. Oligonucleotides **15**–**18** were prepared by standard trityl-off procedures.

Oligonucleotide Purification. Deprotected oligonucleotides containing a 5′-DMT group were suspended in 20% triethylammonium bicarbonate buffer (1.0 M, pH 8.5) in water and injected onto a reverse phase VYDAC 201HS1022 C18 column. Preparative HPLC was performed with a Waters 600E Controller and 994 Diode Array Detecter, using the following gradient: 0–100% B over 50 min, where A = 0.1 M triethylamine acetate, 2% acetonitrile; B = 0.05 M triethylamine acetate, 50% acetonitrile. The collected peaks were dried in vacuo and further purified using Waters C18 Classic SepPak cartridges. The amount of purified oligonucleotide was determined spectrophotometrically, with ϵ_{260} values given in parentheses: **11** (119800 M^{-1} cm^{-1}), **12** (115150 M^{-1} cm^{-1}), **13** (95900 M^{-1} cm^{-1}), **14** (97250 M^{-1} cm^{-1}).

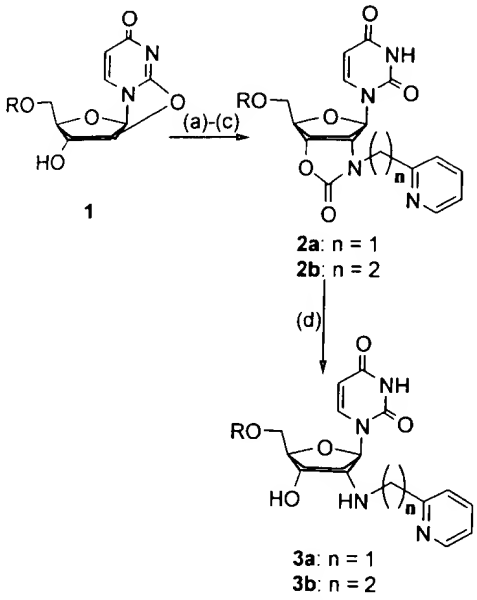
Oligonucleotides **11**, **13**, and **14** were detritylated according to the procedure outlined by ABI manual and desalted using SepPak cartridges (*7*). The detritylated oligonucleotides were further purified using a weak anion exchange column purchased from SynChroPak (AX-100, analytical, semiprep), using the following gradient: 0–90% B over 35 min, where A = 50 mM sodium phosphate (pH 5.9), 30% methanol; B = 50 mM sodium phosphate (pH 5.9), 30% methanol, 1 M ammonium sulfate. The collected peaks were desalted using SepPak cartridges and assayed as described above.

Oligonucleotide Yield. The overall yield of each modified oligonucleotide, following HPLC purification and workup, ranged from 30 to 40%, based on a 12-μmol synthesis. The detritylated, purified oligonucleotides **11**–**14** were characterized by MALDI-TOF mass spectrometry: **11**, calculated, 3355 [M], found, 3356.24 $[M - H]^-$; **12**, calculated, 3408 [M], found, 3407.17 $[M-H]^-$; **13**, calculated, 3230 [M], found, 3228.38 $[M - H]^-$; **14**, calculated, 3320 [M], found, 3319.43 $[M - H]^-$.

Enzymatic Digestion of Oligonucleotides and HPLC Analysis. Approximately 10–30 nmol of purified oligonucleotide was subjected to enzymatic digestion analysis. The digest cocktail (55 μL/sample) contained bacterial alkaline phosphatase (4 μL, 10 μL/unit) and snake venom phosphodiesterase (2.4 μL, 1 mL/mg), in 1 M $MgCl_2$ (0.8 μL), 0.5 M Tris buffer, pH 7.5 (3.5 μL). The reaction mixture was incubated at 37 °C for 8–16 h. The reaction was stopped by adding 3 M sodium acetate (7 μL) and ethanol (155 μL) to the samples, which were then frozen on dry ice (10 min) and centrifuged (10 min) at 4 °C. The supernatants were removed and transferred to new tubes, each containing 452 μL of ethanol. The samples were frozen and centrifuged; the resulting supernatants were removed and dried in vacuo. The samples were dissolved in water (200 μL) and injected onto a reverse phase Vydac (201HS54 4.6 mm × 25 cm, 5 μm, 90 Å) or Prism (Keystone Scientific, 4.6 × 250 mm, 5 μm, 100 Å) C18 column. The product nucleosides were eluted within 20 min, according to either of the following gradients: (Vydac) 0–30% B over 20 min then 30–100% B over 10 min, where A = 0.1 M triethylamine acetate, 2% acetonitrile, B = 0.05 M triethylamine acetate, 50% acetonitrile; (Prism) 0–17% B over 15 min then 17–75% B over 18 min, where A = 0.1 M triethylamine acetate, pH 7.0, 2% acetonitrile, B = 100% acetonitrile. The resulting peaks were compared against the appropriate set of nucleoside standards for a given oligonucleotide sequence.

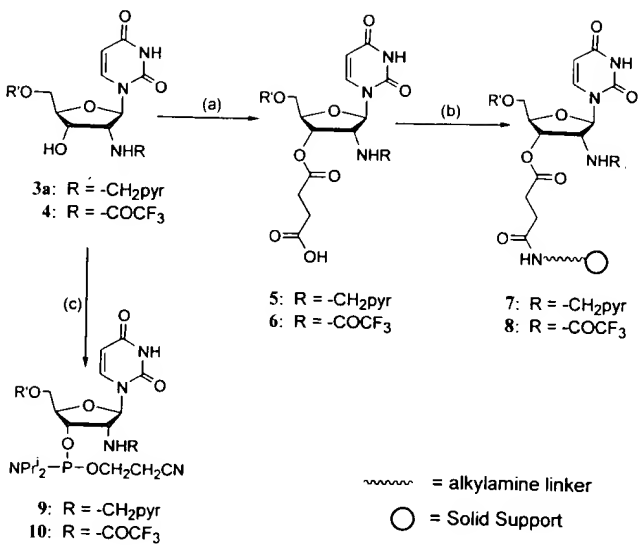
Thermal Denaturation Measurements. Individual oligonucleotides were hybridized to their complementary strands in 50 mM NaP_i buffer (pH 7.0), 0.5 M NaCl, to give solutions that were 2.7 μM in each strand. The samples were heated for 20 min at 70 °C and cooled to 4 °C overnight. Thermal denaturation profiles were measured at 260 nm with an Hewlett-Packard diode array UV–vis spectrophotometer equipped with a Peltier temperature controller and interfaced with a personal computer. Samples were equilibrated at 20 °C for 10–20 min prior to data collection. Absorbance values were taken over a temperature range of 20–70 °C, with measurements made every 0.5 °C with an equilibration time of 60 s for each point. Each hybrid went through 2–4

Scheme 1[a]



[a] Reagents: (a) $(imid)_2CO$, pyridine, rt, 30 h; (b) NH_2R (R = CH_2pyr, CH_2CH_2pyr), DIEA, CH_2Cl_2, rt, 60 h; (c) DBU, THF, reflux, 46 h; (d) 6 N NaOH, dioxane, CH_3OH, 50 °C, 36 h. Abbreviation: R = 4,4′-dimethoxytrityl.

Scheme 2[a]



[a] Reagents: (a) succinic anhydride, pyridine, DMAP, rt, 16 h; (b) solid support, TEA, HOBT, BOP, CH_2Cl_2, rt, 16 h; acetic anhydride, *N*-methylimidazole, pyridine, rt, 12 h; (c) amidite, DIEA, CH_2Cl_2, rt, 50 min. Abbreviation: R′ = 4,4′-dimethoxytrityl.

separate heat-cool cycles, and the T_m values obtained from these heating and cooling traces were averaged to give the final T_m value. Standard deviations were calculated for each duplex.

RESULTS

Synthesis of 2′-Modified Nucleosides. Nucleosides with bidentate amine groups such as aminomethylpyridine (AMPy) (*8*) and aminoethylpyridine (AEPy) at the 2′ ribose position were prepared as shown in Scheme 1. This approach was based on methods developed recently for incorporating 2′-*N*-alkylamino substituents into nucleosides (*9*). Nucleoside **1** was converted to the 3′-*N*-alkyl carbamate upon prolonged treatment with carbonyl diimidazole in pyridine; this was followed by the addition of AMPy or AEPy in the presence of DIEA and dichloromethane. Subsequent cyclization in THF using the cyclization agent DBU produced either **2a** or **2b** in yields of 20% and 10%, respectively. Prolonged heating of **2a** and **2b** in a basic dioxane–methanol solution caused deprotection at the 2′,3′-positions, giving **3a** and **3b** in high yield (92% and 88%, respectively).

Derivatization of solid supports with 2′-modified nucleosides was achieved according to Scheme 2. Nucleoside **4** was prepared according to previously published procedures (*3a, 5, 10*). Both **3a** and **4** were treated with succinic anhydride to give the corresponding hemisuccinates **5** and **6** in yields of 60% and 74%, respectively (*6, 11*). Solid supports such as controlled pore glass (CPG) containing long-chain alkylamine groups were derivatized with **6** using *p*-nitrophenol and DCC. This method resulted in solid supports with low nucleoside loading. Subsequent attempts to prepare the nucleoside-modified solid supports employed the coupling agent BOP in the presence of HOBT and TEA (*12*). The unreacted amine groups were treated with acetic anhydride, and the nucleoside loadings of the solid supports **7** and **8** were determined by spectrophotometric assay (60 and 52 μmol/g, respectively) (*6*).

The preparation of phosphoramidite derivatives of nucleosides **3a** and **4** relied on standard methods (*3a, 6, 10*). Nucleosides **3a** and **4** were treated with 2-chlorocyanoethyl *N,N*-diisopropylaminophosphoramidite in the presence of DIEA to give **9** and **10** in 57% and 77% yield, respectively (Scheme 2).

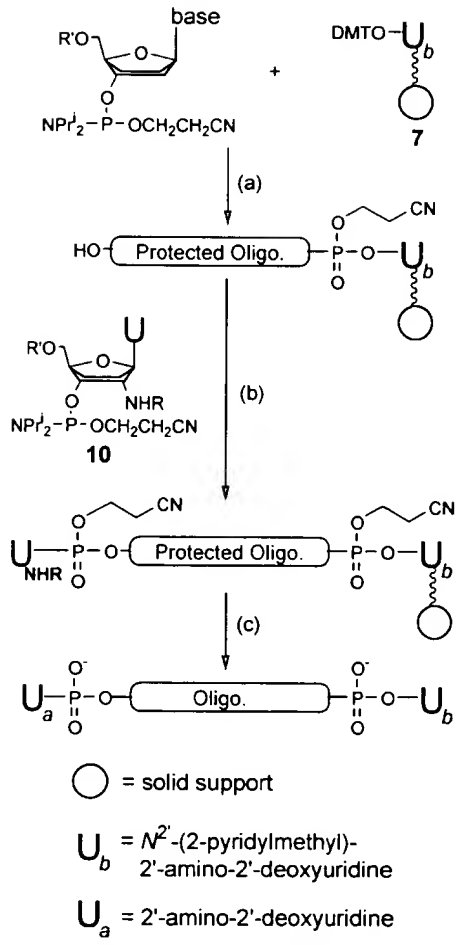
Oligonucleotide Synthesis. A series of oligonucleotides were prepared from the support-bound and phosphoramidite nucleoside derivatives as outlined in Scheme 3 (see Table 1 for sequences). To maximize coupling, the reaction time for the first step was increased from 30 s to 2 min (yield > 95%). The reaction times for **9** and **10** were 15 min in length, leading to coupling yields > 90%.

All oligonucleotides were cleaved from the solid support with concentrated ammonia as a part of the automated synthesis routine, except in the case of **14**, which was manually cleaved. The yield of purified oligonucleotide for **11**–**14** ranged from 30 to 40%, which was comparable to those values observed for **15**–**18**. Results from MALDI-TOF mass spectrometry on **11**–**14** were in excellent agreement with the calculated values. Further characterization of these oligonucleotides was achieved by enzymatic digestion with alkaline phosphatase and phosphodiesterase (Figure 2) (*7*). Analysis of the digestion products showed the expected distribution of nucleosides determined for each oligonucleotide sequence (*13*).

Thermal Denaturation Studies. We investigated the thermal stability of duplexes containing 2′-modified nucleosides. Table 2 shows the transition melting temperatures (T_m) for each duplex prepared in 50 mM sodium phosphate buffer (pH 7.0) containing 0.5 M sodium chloride. The T_m of the 11-mer duplex formed by the unmodified olignucleotides **15** and **16** was 48 °C. When one 5′-terminal nucleoside from this duplex was substituted with either 2′-amino-2′-deoxyuridine (U_a) or $N^{2'}$-(2-pyridylmethyl)-2′-amino-2′-deoxyuridine (U_b), the T_m value remained unchanged (T_m = 47 °C for duplexes **11**:**15** and **12**:**16**). When both U_a and U_b were incorporated at the 5′ ends of the same duplex (**11**:**12**), the T_m value decreased to 45 °C.

The small change in the melting profile was similar to the results obtained with duplexes of identical length, GC content, and type of nucleoside modification, but of different sequence. This second set of duplexes contained

Scheme 3[a]



[a] Steps in the synthesis of oligonucleotide **14**: (a) detritylation of **7**; monomer coupling; normal synthesis cycle; (b) coupling of **10**; (c) cleavage and deprotection. R = $COCF_3$, R' = 4,4'-dimethoxytrityl.

Table 1. Oligonucleotide Sequences[a]

sequence	abbreviation
5'-$\mathbf{U_a}$CAGCTGTAGA	**11**
5'-$\mathbf{U_b}$CTACAGCTGA	**12**
5'-$\mathbf{U_a}$CTCCTACACU$_a$	**13**
5'-$\mathbf{U_a}$CTCCTACACU$_b$	**14**
5'- TCTACAGCTGA	**15**
5'- TCAGCTGTAGA	**16**
5'- TCTCCTACACT	**17**
5'- AGTGTAGGAGA	**18**

[a] The symbol $\mathbf{U_a}$ denotes 2'-amino-2'-deoxyuridine; $\mathbf{U_b}$ denotes $N^{2'}$-(2-pyridylmethyl)-2'-amino-2'-deoxyuridine.

2'-modified nucleosides at both the 3' and 5' ends of the same strand. Duplex **13:18** contained $\mathbf{U_a}$ nucleosides at the 5' and 3' termini, whereas duplex **14:18** contained $\mathbf{U_a}$ at the 5' end and $\mathbf{U_b}$ at the 3' end. The T_m values (46 °C) for duplexes **13:18** and **14:18** decreased slightly in comparison to the value of the unmodified duplex **17:18** (T_m = 48 °C; Figure 3).

DISCUSSION

Synthetic Strategy. Two general methods exist for preparing oligonucleotides containing transition metal complexes. One method involves the synthesis of nucleosides that possess metal-binding ligands, followed by incorporation of these modified nucleosides into oligonucleotides and subsequent metal complexation at the specific attachment sites (*3e, 14*). The second method

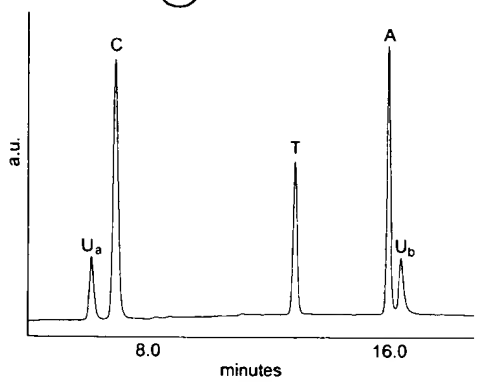


Figure 2. Products of enzymatic digestion of **14** as analyzed by reverse-phase HPLC.

Table 2. Thermal Denaturation Temperatures for Oligonucleotides Containing 2'-Substituted Nucleosides

duplex	T_m[a] (°C)	modification[b]
15:16	48.2 ± 0.5	none
11:15	46.6 ± 0.4	5' $\mathbf{U_a}$
12:16	46.7 ± 0.4	5' $\mathbf{U_b}$
11:12	45.0 ± 0.5	5' $\mathbf{U_a}$, 5' $\mathbf{U_b}$
17:18	47.6 ± 0.2	none
13:18	45.8 ± 0.5	5' $\mathbf{U_a}$, 3' $\mathbf{U_a}$
14:18	46.2 ± 0.5	5' $\mathbf{U_a}$, 3' $\mathbf{U_b}$

[a] Values determined in 50 mM NaP_i buffer (pH 7.0) containing 0.5 M NaCl. The concentration of each oligomer was 2.7 μM. [b] The symbol $\mathbf{U_a}$ denotes 2'-amino-2'-deoxyuridine; $\mathbf{U_b}$ denotes $N^{2'}$-(2-pyridylmethyl)-2'-amino-2'-deoxyuridine.

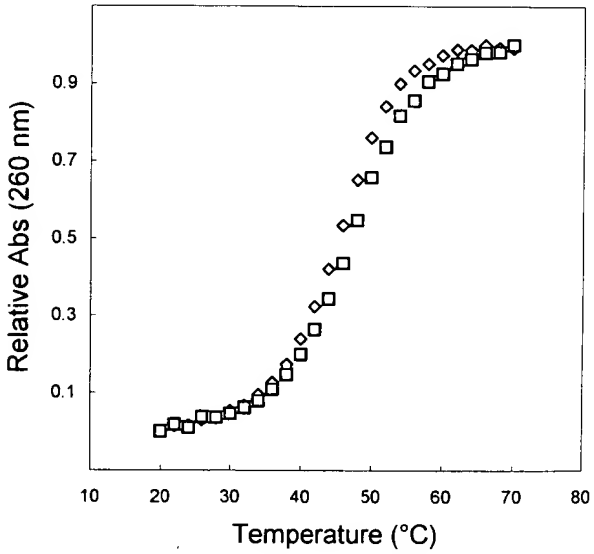


Figure 3. Thermal denaturation curves for the unmodified duplex **17:18** (□) and modified duplex **14:18** (◇).

entails the synthesis of metal-containing nucleosides that are incorporated during solid-phase oligonucleotide synthesis (*4ab, 15*). The 2'-modified nucleosides described in this work can be used in either method, resulting in oligonucleotides site-specifically labeled with metal complexes (*3e, 4*).

The choice of 2'-substituents described here are based on ligands that avidly bind transition metal complexes, such as AMPy and AEPy (*16*). The site-specific labeling of oligonucleotides with metal reagents is of considerable interest for several reasons. Recent experiments involving ruthenium-modified duplexes have shown that DNA can mediate energy and electron transfer reactions (*3e, 15f, 17*). Additionally, metal-modified primers have been used in dideoxy DNA sequencing techniques and elec-

trochemistry-based DNA assays (*18*). Therefore, nucleosides containing metal-binding ligands such as those in Figure 1 can facilitate the use of metal complexes as probes of nucleic acid structure and function (*3e, 4*).

Synthesis of 2′-Modified Nucleosides. The synthesis of nucleosides containing 2′-*N*-alkylamino substituents is an extension of methods developed by Sebesta, McGee, and co-workers (*9*). The yields of isolation determined for **2a** and **2b** are lower than those observed for nucleosides containing similarly bulky substituents and may be attributed to the purification conditions required for DMT-protected vs silyl-protected intermediates (*9a*). The products **3a** and **3b** are isolated in yields comparable to the yields reported by Sebesta for similar 2′-modified nucleosides.

The preparation of solid supports derivatized with 2′-modified nucleosides is complicated by the poor steric accessibility of the 3′ ribose site (*19*). The steric bulk at the 2′ position hinders the reaction of the 3′ hydroxyl with succinic anhydride. Succination of **3a** and **4** proceeds in reasonable yields and demonstrates that both small and large functional groups at the 2′ position can be tolerated in the succination step. High nucleoside loadings for **7** and **8** are achieved using the coupling agent BOP and an excess of nucleoside hemisuccinate in the derivatization step. While the solid support employed here is glass-based, the method is applicable to other supports containing long-chain alkylamine linkers.

Successful derivatization of supports with nucleosides such as **3a** and **4** affords the synthesis of 3′ oligonucleotide conjugates in which the label is incorporated *directly* on the ribose ring. This is a significant advance since the current preparation of 3′ oligonucleotide conjugates is achieved with supports containing either nucleosides with *base-tethered* primary amine groups (*20*) or *nonnucleosidic* amine derivatives (*21*). The ribose is an attractive attachment site since the labels introduced here cause fewer perturbations to the secondary duplex structure than labels attached to the nucleoside base (*4*). Additionally, the absence of a long linker between the attachment site and the incorporated label minimizes disruptions to the hydrogen bonding capacity of the oligonucleotide conjugate.

Oligonucleotide Synthesis with 2′-Modified Nucleosides. The successful large-scale synthesis of several 11-mer oligonucleotides validates the utility of 2′-modified nucleosides as support-bound and phosphoramidite derivatives (Table 1). Oligonucleotide synthesis beginning with **7** or **8** proceeds with minor modification to the automated protocol. The coupling yields of phosphoramidites **9** and **10** are acceptable for routine oligonucleotide preparation, and the purification of the several 11-mer modified oligonucleotides is straightforward. The isolation yields for **11**−**14** are comparable to values determined for oligonucleotides **15**−**18** under identical synthetic and purification conditions, implying that the use of 2′-modified nucleosides does not compromise the overall yield.

Effect of 2′-Modified Nucleosides on Duplex Stability. The presence of nucleosides containing ribose substituents at the 2′ position causes slight destabilization to the modified duplexes, as assessed by thermal denaturation studies. Interestingly, analysis of the transition melting (T_m) temperatures listed in Table 2 suggests that the T_m values are influenced by the number of 2′-modified nucleosides present in a duplex, not the size of the 2′ substituent. For example, thermal denaturation of duplexes **11:15** and **12:16** produces identical T_m values, despite the difference in the size of the 2′ substituent (−NH_2 vs AMPy). The T_m of **11:12** shows the effect of placing two 2′-modified nucleosides at the 5′ ends of the duplex. Similar results are obtained with a second set of duplexes identical in length, GC content, and type of nucleoside modification. The placement of two 2′-modified nucleosides at the 5′ and 3′ ends of the same strand results in duplexes destabilized by 1−2 °C (**13:18** and **14:18** vs **17:18**). We conclude that the extent of duplex destabilization is the same when two 2′-modified nucleosides are introduced at either (1) the 5′ and 3′ ends of a one strand hybridized to its complement, or (2) the 5′ ends of complementary strands.

Compared to 2′-*deoxy*nucleosides, 2′-*amino*nucleosides favor the 2′-endo sugar conformation to a higher degree (*22*). This observation suggests that 2′-aminonucleosides should stabilize DNA/DNA duplexes. However, T_m data for a series of 9-mer duplexes containing a 2′-aminonucleoside in the middle of the sequence indicate that the presence of these modified nucleosides has a destabilizing effect (*3a*). Our work shows that placement of the 2′-amino-modified nucleosides at the ends of the duplexes minimizes the destabilization imposed by the altered sugar conformation.

CONCLUSION

We report the synthesis of 2′-modified nucleosides designed specifically for incorporating reporter molecules into oligonucleotides. Because these nucleosides contain modifications to the ribose ring (as opposed to the nucleobase), they are important contributions to current library of modified nucleosides. The introduction of metal-binding ligands at the 2′ position is achieved after two steps. Conversion of these nucleosides to solid support-bound and phosphoramidite derivatives proceeds in good yield. The powerful combination of these derivatives affords the preparation of an entirely new class of oligonucleotide conjugates—those which contain label attachment sites at 3′, intervening, and 5′ sites of a duplex.

Thermal denaturation studies indicate that the presence of 2′-modified nucleosides in 11-mer duplexes has a slight destabilizing effect on the duplex structure. This effect is limited by the selective placement of these nucleosides at the ends of the duplexes. Interestingly, the size of the metal-binding substituent does not influence the magnitude of the destabilization. This characteristic makes these 2′-modified nucleosides even more attractive for use in the site-specific incorporation of reporter molecules into oligonucleotides.

The methodologies employed here can be extended to other modified nucleosides. Succination yields for nucleosides containing metal-binding ligands at locations other than the 2′ position are expected to be much higher due to the absence of steric constraints. While the solid support is glass-based, the method is applicable to other solid supports containing any long-chain alkylamine linker. The library of solid supports containing modified nucleosides can be significantly expanded with the coupling conditions described here. These nucleoside reagents will enable the incorporation of labels known to be useful probes of nucleic acid structure and function.

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Supporting Information Available: MALDI-TOF mass spectra for **11**–**13**. HPLC of digestion products of **11**–**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) (a) Verma, S., and Eckstein, F. (1998) Modified Oligonucleotides: Synthesis and Strategy for Users. *Annu. Rev. Biochem. 67*, 99–134. (b) Eaton, B. E., and Pieken, W. A. (1995) Ribonucleotides and RNA. *Annu. Rev. Biochem. 64*, 837–863. (c) Aurup, H., Williams, D. M., and Eckstein, F. (1992) 2′-Fluoro-2′-deoxynucleoside and 2′-Amino-2′-deoxynucleoside 5′-Triphosphates As Substrates For T7 RNA-Polymerase. *Biochemistry 31*, 9636–9641.

(2) (a) Tuerk, C., and Gold, L. (1990) Systematic Evolution of Ligands By Exponential Enrichment – RNA Ligands to Bacteriophage-T4 Dna-Polymerase. *Science 249*, 505–510. (b) Lin, Y., Qiu, Q., Gill, S. C., and Jayasena, S. D. (1994) Modified Rna Sequence Pools For in-Vitro Selection. *Nucleic Acids Res. 22*, 5229–5234.

(3) (a) Aurup, H., Tuschi, T., Benseler, F., Ludwig, J., and Eckstein, F. (1994) Oligonucleotide duplexes containing 2′-amino-2′-deoxycytidines: thermal stability and chemical reactivity. *Nucleic Acids Res. 22*, 20–24. (b) Sigurdsson, S. T., and Eckstein, F. (1996) Site specific labeling of sugar residues in oligoribonucleotides: Reactions of aliphatic isocyanates with 2′ amino groups. *Nucleic Acids Res. 24*, 3129–3133. (c) Kuznetsova, K. G., Romanova, E. A., Volkov, E. M., Tashlitsky, V. N., Oretskaya, T. S., Krynetskaya, N. F., and Shabarova, Z. A. (1993) *J. Bioorg. Chem. (Moscow) 19*, 455–466. (d) Sigurdsson, S. T., Tuschl, T., and Eckstein, F. (1995) Probing RNA Tertiary Structure – Interhelical Cross-Linking of the Hammerhead Ribozyme. *RNA-Publ. RNA Soc. 1*, 575–583. (e) Meade, T. J., and Kayyem, J. F. (1995) Electron Transfer through DNA: Site-Specific Modification of Duplex DNA with Ruthenium Donors and Acceptors. *Angew. Chem., Int. Ed. Engl. 34*, 352–353.

(4) (a) Krider, E. S., Rack, J. J., Frank, N. L., and Meade, T. J. (2001) Automated Synthesis of 3′-Metalated Oligonucleotides. *Inorg. Chem. 40*, 4002–4009. (b) Rack, J. J., Krider, E. S., and Meade, T. J. (2000) Spectroscopy and Electrochemistry of Ruthenium-Modified Nucleic Acids: Design of a Novel Metal-Binding Nucleoside. *J. Am. Chem. Soc. 122*, 6287–6288.

(5) McGee, D., Vaughn-Settle, A., Vargeese, C., and Zhai, Y. (1996) 2′-Amino-2′-deoxyuridine via an intramolecular cyclization of a trichloroacetimidate. *J. Org. Chem. 61*, 781–785.

(6) Gait, M., Ed. (1984) *Oligonucleotide Synthesis: A Practical Approach*, Oxford University Press, Oxford.

(7) (1992) *Evaluating and Isolating Synthetic Oligonucleotides: The Complete Guide*, Perkin-Elmer/Applied Biosystems Division, Publication no. 239902, Foster City, CA.

(8) Abbreviations: AMPy, aminomethylpyridine; AEPy, aminoethylpyridine; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethanolamine; DMAP, (dimethylamino)pyridine; DMT, 4,4′-dimethoxytrityl; EtOAC, ethyl acetate; HOBT, hydroxybenzotriazole; MALDI-TOF, matrix-assisted laser desorption-time-of-flight; TEA, triethylamine; THF, tetrahydrofuran.

(9) (a) Sebesta, D. P., O'Rourke, S. S., Martinez, R. L., Pieken, W. A., and McGee, D. P. C. (1996) 2′-Deoxy-2′-alkoxylaminouridines: Novel 2′-substituted uridines prepared by intramolecular nucleophilic ring opening of 2, 2′−O-anhydrouridines. *Tetrahedron 52*, 14385–14402. (b) McGee, D. P. C., Sebesta, D. P., O'Rourke, S. S., Martinez, R. L., Jung, M. E., and Pieken, W. A. (1996) Novel nucleoside va intramolecular functionalization of 2,2′-anhydrouridine derivatives. *Tetrahedron Lett. 37*, 1995–1998.

(10) Pieken, W. A., Olsen, D. B., Benseler, F., Aurup, H., and Eckstein, F. (1991) Kinetic characterization of ribonuclease-resistant 2′-modified hammerhead ribozymes. *Science 253*, 314–317.

(11) Caruthers, M. H., Barone, A. D., Beaucage, S. L., Dodds, D. R., Fisher, E. F., McBride, L. J., Matteucci, M., Stabinsky, Z., and Tang, J.-Y. (1987) In *Methods in Enzymology: Recombinant DNA* (R. W. a. L. Grossman, Ed.) vol. 154, pp 287–313, Academic Press, Inc., New York

(12) Knorr, R., Trzeciak, A., Bannwarth, W., and Gillessen, D. (1989) New coupling reagents in peptide chemistry. *Tetrahedron Lett. 30*, 1927–1930.

(13) Supporting data can be found in the Supporting Information.

(14) (a) Ledoan, T., Perrouault, L., Chassignol, M., Thuong, N. T., and Helene, C. (1987) Sequence-Targeted Chemical Modifications of Nucleic-Acids By Complementary Oligonucleotides Covalently Linked to Porphyrins. *Nucleic Acids Res. 15*, 8643–8659. (b) Bannwarth, W., Schmidt, D., Stallard, R. L., Hornung, C., Knorr, R., and Muller, F. (1988) Bathophenanthroline-Ruthenium(Ii) Complexes As Non-Radioactive Labels For Oligonucleotides Which Can Be Measured By Time-Resolved Fluorescence Techniques. *Helv. Chim. Acta 71*, 2085–2099. (c) Chen, C. H. B., and Sigman, D. S. (1988) Sequence-Specific Scission of Rna By 1,10-Phenanthroline Copper Linked to Deoxyoligonucleotides. *J. Am. Chem. Soc. 110*, 6570–6572. (d) Telser, J., Cruickshank, K. A., Schanze, K. S., and Netzel, T. L. (1989) DNA oligomers and duplexes containing a covalently attached derivative of tris(2,2′-bipyridine)ruthenium(II) – Synthesis and characterization by thermodynamic and optical spectroscopic measurements. *J. Am. Chem. Soc. 111*, 7221–7226. (e) Modak, A. S., Gard, J. K., Merriman, M. C., Winkeler, K. A., Bashkin, J. K., and Stern, M. K. (1991) Toward chemical ribonucleases II. Synthesis and characterization of nucleoside-bipyridine conjugates – hydrolytic cleavage of RNA by their copper(II) complexes. *J. Am. Chem. Soc. 113*, 283–291. (f) Jenkins, Y., and Barton, J. K. (1992) A sequence-specific molecular light switch – tethering of an oligonucleotide to a dipyridophenazine complex of ruthenium(II). *J. Am. Chem. Soc. 114*, 8736–8738. (g) Bashkin, J. K., Frolova, E. I., and Sampath, U. S. (1994) Sequence-specific cleavage of HIV messenger-RNA by a ribozyme mimic. *J. Am. Chem. Soc. 116*, 5981–5982. (h) Arkin, M. R., Stemp, E. D. A., Pulver, S. C., and Barton, J. K. (1997) Long-range oxidation of guanine by Ru(III) in duplex DNA. *Chem., Biol. 4*, 389–400. (i) Inoue, H., Furukawa, T., Shimizu, R., Tamura, T., Matsui, M. N., and Ohtsuka, E. (1999) Efficient site specific cleavage of RNA using a terpyridine–copper(II) complex joined to a 2′-O-methyloligonucleotide by a nonflexible linker. *Chem. Commun.*, 45–46. (j) Wiederholt, K., and McLaughlin, L. W. (1999) A 2,2′-bipyridine ligand for incorporation into oligodeoxynucleotides: synthesis, stability and fluorescence properties of ruthenium-DNA complexes. *Nucleic Acids Res. 27*, 2487–2493. (k) Ortmans, I., Content, S., Boutonnet, N., Kirsch-De Mesmaeker, A., Bannwarth, W., Constant, J. F., Defrancq, E., and Lhomme, J. (1999) Ru-labeled oligonucleotides for photoinduced reactions on targeted DNA guanines. *Chem. Eur. J. 5*, 2712–2721. (l) Holmlin, R. E., Dandliker, P. J., and Barton, J. K. (1999) Synthesis of metallointercalator-DNA conjugates on a solid support. *Bioconjugate Chem. 10*, 1122–1130. (m) Khan, S. I., and Grinstaff, M. W. (1999) Palladium(0)-catalyzed modification of oligonucleotides during automated solid-phase synthesis. *J. Am. Chem. Soc. 121*, 4704–4705.

(15) (a) Bannwarth, W., and Schmidt, D. (1989) A simple specific labeling for oligonucleotides by bathophenanthroline-Ru(II) complexes as nonradioactive label molecules. *Tetrahedron Lett. 30*, 1513–1516. (b) Manchanda, R., Dunham, S. U., and Lippard, S. J. (1996) Automated solid-phase synthesis of site-specifically platinated oligodeoxyribonucleotides. *J. Am. Chem. Soc. 118*, 5144–5145. (c) Schliepe, J., Berghoff, U., Lippert, B., and Cech, D. (1996) Automated solid-phase synthesis of platinated oligonucleotides via nucleoside phosphonates. *Angew. Chem., Int. Ed. 35*, 646–648. (d) Magda, D., Crofts, S., Lin, A., Miles, D., Wright, M., and Sessler, J. L. (1997) Synthesis and kinetic properties of ribozyme analogues prepared using phosphoramidite derivatives of dysprosium(III) texaphyrin. *J. Am. Chem. Soc. 119*, 2293–2294. (e) Meggers, E., Kusch, D., and Giese, B. (1997) An

efficient synthesis of enantiomerically pure Delta- and Lambda-ruthenium(II)-labeled oligonucleotides. *Helv. Chim. Acta 80*, 640–652. (f) Hurley, D. J., and Tor, Y. (1998) Metal-containing oligonucleotides: Solid-phase synthesis and luminescence properties. *J. Am. Chem. Soc. 120*, 2194–2195. (g) Khan, S. I., Beilstein, A. E., Smith, G. D., Sykora, M., and Grinstaff, M. W. (1999) Synthesis and excited-state properties of a novel ruthenium nucleoside: 5-[Ru(bpy)(2)(4-m-4′-pa-bpy)](2+)-2′-deoxyuridine. *Inorg. Chem. 38*, 2411–2415. (h) Khan, S. I., Beilstein, A. E., Sykora, M., Smith, G. D., Hu, X., and Grinstaff, M. W. (1999) Automated solid-phase DNA synthesis and photophysical properties of oligonucleotides labeled at the 5′-terminus with Ru(bpy)(3)(2+). *Inorg. Chem. 38*, 3922–3925. (i) Lewis, F. D., Helvoigt, S. A., and Letsinger, R. L. (1999) Synthesis and spectroscopy of Ru(II)-bridged DNA hairpins. *Chem. Commun.*, 327–328. (j) Hu, X., Smith, G. D., Sykora, M., Lee, S. J., and Grinstaff, M. W. (2000) Automated solid-phase synthesis and photophysical properties of oligodeoxynucleotides labeled at 5′-aminothymidine with Ru(bpy)-(2)(4-m-4′-cam-bpy)(2+). *Inorg. Chem. 39*, 2500–2504.

(16) (a) Brown, G. M., Weaver, T. R., Keene, F. R., and Meyer, T. J. (1976) Oxidation of Coordinated Diamines in Bis(2,2′-bipyridine) Complexes of Ruthenium. *Inorg. Chem. 15*, 190–196. (b) Juris, A., Balzani, V., Barigelletti, F., Campagna, S., Belser, P., and Vonzelewsky, A. (1988) Ru(II) polypyridine complexes – photophysics, photochemistry, electrochemistry, and chemiluminescence. *Coord. Chem. Rev. 84*, 85–277.

(17) (a) Fu, P. K. L., and Turro, C. (1999) Energy transfer from nucleic acids to Tb(III): Selective emission enhancement by single DNA mismatches. *J. Am. Chem. Soc. 121*, 1–7. (b) Bannwarth, W., Pfleiderer, W., and Muller, F. (1991) Energy-transfer within oligonucleotides from a lumazine (= pteridine-2,4(1H,3H)-dione) chromophore to bathophenanthroline-ruthenium(II) complexes. *Helv. Chim. Acta 74*, 1991–1999. (c) Bannwarth, W., and Muller, F. (1991) Energy-transfer from a lumazine (= pteridine-2,4(1H,3H)-dione) chromophore to bathophenanthroline-ruthenium(II) complexes during hybridization processes of DNA. *Helv. Chim. Acta 74*, 2000–2008. (d) Holmlin, R. E., Dandliker, P. J., and Barton, J. K. (1997) Charge transfer through the DNA base stack. *Angew. Chem., Int. Ed. 36*, 2715–2730. (e) Fukui, K., and Tanaka, K. (1998) Distance dependence of photoinduced electron transfer in DNA. *Angew. Chem., Int. Ed. 37*, 158–161. (f) Okahata, Y., Kobayashi, T., Tanaka, K., and Shimomura, M. (1998) Anisotropic electric conductivity in an aligned DNA cast film. *J. Am. Chem. Soc. 120*, 6165–6166. (g) Harriman, A. (1999) Electron tunneling in DNA. *Angew. Chem., Int. Ed. 38*, 945–949. (h) Kelley, S. O., and Barton, J. K. (1999) Electron transfer between bases in double helical DNA. *Science 283*, 375–381. (i) Tierney, M. T., Sykora, M., Khan, S. I., and Grinstaff, M. W. (2000) Photoinduced electron transfer in an oligonucleotide duplex: observation of the electron-transfer intermediate. *J. Phys. Chem. B 104*, 7574–7576. (j) Lewis, F. D., Wu, T. F., Liu, X. Y., Letsinger, R. L., Greenfield, S. R., Miller, S. E., and Wasielewski, M. R. (2000) Dynamics of photoinduced charge separation and charge recombination in synthetic DNA hairpins with stilbenedicarboxamide linkers. *J. Am. Chem. Soc. 122*, 2889–2902.

(18) (a) Bannwarth, W. (1989) Bathophenanthroline Ru(II) complexes as nonradioactive labels for dideoxy DNA sequencing. *Analytical Biochemistry 181*, 216–219. (b) Yu, C. J., Yowanto, H., Wan, Y. J., Meade, T. J., Chong, Y., Strong, M., Donilon, L. H., Kayyem, J. F., Gozin, M., and Blackburn, G. F. (2000) Uridine-conjugated ferrocene DNA oligonucleotides: Unexpected cyclization reaction of the uridine base. *J. Am. Chem. Soc. 122*, 6767–6768.

(19) Goodchild, J. (1990) Conguates of oligonucleotides and modified oligonucleotides: A review of their synthesis and properties. *Bioconjugate Chem. 1*, 165–191.

(20) Ueno, Y., Mikawa, M., and Matsuda, A. (1998) Nucleosides and nucleotides. 170. Synthesis and properties of oligodeoxynucleotides containing 5-[*N*-[2-[*N*,*N*-bis(2-aminoethyl)amino]-ethyl]carbamoyl]-2′-deoxyuridine and 5-[*N*-[3-[*N*,*N*-bis(3-aminopropyl)amino]propyl]carbamoyl]-2′-deoxyuridine. *Bioconjugate Chem. 9*, 33–39.

(21) (a) Haralambidis, J., Duncan, L., Angus, K., and Tregear, G. W. (1990) The synthesis of polyamide oligonucleotide conjugate molecules. *Nucleic Acids Res. 18*, 493–499. (b) Asseline, U., and Thuong, N. T. (1990) New solid-phase for automated synthesis of oligonucleotides containing an aminoalkyl linker at their 3′ end. *Tetrahedron Lett. 31*, 81–84. (c) Reed, M. W., Adams, A. D., Nelson, J. S., and Meyer, R. B. (1991) Acridine-derivatized and cholesterol-derivatized solid supports for improved synthesis of 3′-modified oligonucleotides. *Bioconjugate Chem. 2*, 217–225. (d) Petrie, C. R., Reed, M. W., Adams, A. D., and Meyer, R. B. (1992) An Improved Cpg Support For the Synthesis of 3′-Amine-Tailed Oligonucleotides. *Bioconjugate Chem. 3*, 85–87. (e) Thaden, J., and Miller, P. S. (1993) Automated Synthesis of Oligodeoxyribonucleoside Methylphosphonates Having [*N*-(3-Aminoprop-1-yl)-*N*-(2-hydroxyethyl)-2-aminoethyl] Phosphate or Methylphosphonic Acid At the 3′ End Using a Modified Controlled-Pore Glass Support. *Bioconjugate Chem. 4*, 395–401. (f) Nelson, P. S., Frye, R. A., and Liu, E. (1989) Bifunctional oligonucleotide probes synthesized using a novel CPG support are able to detect single base pair mutations. *Nucleic Acids Res. 17*, 7187–7194. (g) Nelson, P. S., Kent, M., and Muthini, S. (1992) Oligonucleotide labeling methods III: direct labeling of oligonucleotides Employing a novel, nonnucleosidic, 2-aminobutyl-1,3-propanediol backbone. *Nucleic Acids Res. 20*, 6253–6259. (h) Hovinen, J., Guzaev, A., Azhayeva, E., Azhayev, A., and Lonnberg, H. (1995) Imidazole tethered oligodeoxyribonucleotides: Synthesis and RNA cleaving activity. *J. Org. Chem. 60*, 2205–2209. (i) Mullah, B., and Andrus, A. (1997) Automated synthesis of double dye-labeled oligonucleotides using tetramethylrhodamine (TAMRA) solid supports. *Tetrahedron Lett. 38*, 5751–5754. (j) Mullah, B., Livak, K., Andrus, A., and Kenney, P. (1998) Efficient synthesis of double dye-labeled oligodeoxyribonucleotide probes and their application in a real time PCR assay. *Nucleic Acids Res. 26*, 1026–1031. (k) McMinn, D. L., Matray, T. J., and Greenberg, M. M. (1997) Efficient solution phase synthesis of oligonucleotide conjugates using protected biopolymers containing 3′-terminal alkylamines. *J. Org. Chem. 62*, 7074–7075. (l) McMinn, D. L., and Greenberg, M. M. (1998) Postsynthetic conjugation of protected oligonucleotides containing 3′-alkylamines. *J. Am. Chem. Soc. 120*, 3289–3294. (m) Kahl, J. D., and Greenberg, M. M. (1999) Solution-phase bioconjugate synthesis using protected oligonucleotides containing 3′-alkyl carboxylic acids. *J. Org. Chem. 64*, 507–510.

(22) Benseler, F., Williams, D., and Eckstein, F. (1992) Synthesis of suitably protected phosphoramidites of 2′-fluoro-2′-deoxyguanosine and 2′-Amino-2′-deoxyguanosine for incorporation into oligoribonucleotides. *Nucleosides Nucleotides 11*, 1333–1351.

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